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Short communication

The presence of gamma-hydroxybutyric acid (GHB) and gamma-butyrolactone (GBL) in alcoholic and non-alcoholic beverages

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Abstract

Gamma-hydroxybutyric acid (GHB) and its precursor gamma-butyrolactone (GBL) are regularly implicated in instances of surreptitious drug administration, particularly in beverages (so-called "spiked drinks"). In order to assist in the interpretation of cases where analysis of the actual beverage is required, over 50 beverages purchased in the UK were analysed for the presence of GHB and GBL. It was found that naturally occurring GHB and GBL were detected in those beverages involving the fermentation of white and particularly red grapes. No GHB or GBL was detected in other drinks such as beer, juice, spirits or liqueurs. GHB/GBL was detected in red wine vermouth (8.2 mg/L), sherry (9.7 mg/L), port (GBL), red wine (4.1–21.4 mg/L) and white wine (<3–9.6 mg/L). The presence of GHB/GBL did not appear to be influenced by the alcohol content or the pH of the beverage. In addition, the concentration in wines did not appear to be related to the geographical origin of the grape type. This is believed to be the first published data concerning the endogenous presence of GHB and GBL in the beverages described.

Keywords: Gamma-hydroxybutyric acid; Gamma-butyrolactone; Beverages

1. Introduction

Gamma-hydroxybutyric acid (GHB), also known as "Liquid Ecstasy" has been implicated in many cases of suspected surreptitious administration, potentially for the purpose of increasing victim vulnerability to sexual assault [1]. Due to the rapid dissolving properties of GHB powder resulting in a colourless solution when in water, the perceived route of administration is typically associated with the adulteration of a victim's beverage. The precursor solvent gamma-butyrolactone (GBL) may be used as an alternative as it is rapidly converted to GHB in the body [2]. The use of these compounds in this way can be a very dangerous practice as the exact concentration of the resultant solution is largely unknown and may result in serious and potentially fatal GHB intoxication. This would particularly be the case if the "spiked" beverage was alcoholic or if the victim was/had been drinking alcohol prior to the incident. Following an accusation of suspected "spiked drink", as part of the investigation and if available, the actual beverage may be analysed to determine the presence of drugs (e.g. GHB). The necessary dose of GHB to produce incapacitation or even disinhibition (approximately >2 g) would be associated with high concentrations in beverages due to the relatively small liquid volume of most drinks. Nonetheless, it would be necessary to be aware of any naturally occurring GHB or GBL in the beverage. GHB is believed to be present in ripe fruits and meat and Collison et al. recently presented data

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from the United States for various beverages and foodstuffs [3,4]. It was found that GHB was present at its highest concentration in red (2.00–23.00 mg/L) and white wine (0.65–9.53 mg/L), followed by vinegars (0.83–11.25 mg/L), soy sauce (2.79–18.10 mg/L), liqueurs (<0.25–4.20 mg/L), various non-distilled drinks (1.88–6.68 mg/L), beer (<0.25–2.10 mg/L) and coffee (0.52–2.09 mg/L). No GHB was detected (<0.50 mg/L) in any of the distilled alcoholic drinks (e.g. whisky) or juices tested (except grape juice). There are currently no available or published data regarding specific concentrations in alcoholic and non-alcoholic beverages commercially available in the UK.

Another aspect of the necessity of information relating to the natural presence of GHB in drinks involves the increasing availability of alleged detection kits for drugs (including GHB) in beverages. These products are marketed for individuals to be able to "test" their drink to determine whether a drug has been added. Apart from the immediate flaws in the process (i.e. only detect a limited number of compounds), additional problems include: (i) it may be "negative" at the time of testing but may require continual testing, (ii) the chemistry involved in detection may lead to false positive/negative results and (iii) the lack of any immediate confirmation [5,6]. It is also conceivable that depending on the nature or sensitivity of the assay, the presence of certain residual/endogenous compounds may also lead to false positive findings. The possibility of false positive or negative results could have important medical, social and legal ramifications. Therefore, there is both a forensic and public requirement for data concerning the presence of compounds, in particular GHB, in alcoholic and non-alcoholic beverages. This short communication presents novel data from a study of over 50 beverages purchased within the UK.

2. Materials and methods

Beverages were obtained from various licensed premises and stores. In some cases multiple products/brands were analysed of the same drink group (e.g. lager). A 5 mL aliquot of each beverage was retained and stored at 4 °C prior to analysis (typically analysed within 3 days following collection). Samples were initially analysed using gas chromato-

Table 1
Presence of GHB/GBL in various beverages

Beverage	Alcohol content (%, v/v)	GHB concentration (mg/L)	GBL detected? (LOD = 5 mg/L)
Aftershock blue	40	ND	No
Amaretto	28	ND	No
Bitter	4	ND	No
Cream liqueur	17	ND	No
Bourbon	40	ND	No
Brandy	40	ND	No
Cider	5.5	ND	No
Drambuie	40	ND	No
Gin	40	ND	No
Red wine vermouth	17	8.2	Yes
Dark rum	40	ND	No
White rum	37.5	ND	No
Sherry	17.5	9.7	Yes
Vodka	37.5	ND	No
Vodka + juice	5	ND	No
Vodka schnapps	4.4	ND	No
Whisky	40	ND	No
Cranberry juice	None	ND	No
Grapefruit juice	None	ND	No
Orange juice	None	ND	No
Pineapple juice	None	ND	No
Tomato juice	None	ND	No
Tonic water	None	ND	No
Tequila	38	ND	No
Absinthe	70	ND	No
Lager	4	ND	No
Port	20	ND	Yes
Red grape juice	None	ND	No
White grape juice	None	ND	No
Red wine	12–14	4.1–21.4 (mean 12.6)	Yes
White wine	9.5–13.5	<3–9.6 (mean 5.7)	Yes

LOD: limit of detection; ND: not detected (GHB limit of detection = 3 mg/L).

graphy with flame ionization detection (GC–FID). Gas chromatography with mass spectrometry (GC–MS) was used for confirmation of "positive" GC–FID samples in addition to some randomly selected beverages.

2.1. Chromatographic methods

GC–FID and GC–MS analyses were performed using previously published methods [7,8]. In summary; GC–FID detected the presence of GHB and GBL as "total GBL" as it involved complete conversion to GBL prior analysis. This involved acidification with 6 M H_2SO_4 and extraction with chloroform, including 100 mg/L hexanoic acid as an internal standard. GC–MS detected the presence of GHB following trimethylsilane (TMS) derivatisation and analysis in the full scan mode. GHB-D_6 was included as an internal standard (in 0.05 M H_2SO_4) followed by extraction with acetonitrile.

2.2. Beverage analysis

GHB calibration standards of 1, 2.5, 5, 10 mg/L were prepared in blank equine plasma (pre-screened <1 mg/L GHB) along with an internal quality control standard of 7.5 mg/L. Plasma presented less derivatisation problems for GC–MS compared to the use of aqueous standards (e.g. water) and had previously been shown to be valid for the analysis of more aqueous specimens (e.g. urine) [9].

Beverages were directly extracted for GC–FID analysis; however, they required three-fold dilution with blank equine plasma prior to GC–MS analysis. This was due to observed chromatographic and mass spectral interferences/extraneous peaks following TMS derivatisation of undiluted/non-matrix matched samples (in particular, wine). Such interferences did not occur when using plasma. Consequently, this dilution factor reduced the sensitivity of the GC–MS assay from 1 to 3 mg/L.

The pH of the red and white wines were measured using a Jenway pH Meter 3310 (Dunmow, Essex, UK).

3. Results and discussion

Table 1 shows GHB/GBL was detected in only a few of the non-alcoholic and alcoholic beverages analysed in this study, primarily those involving grapes and fermentation processes in their production such as wine (GHB <3–21 mg/L), port (GBL) and sherry (GHB 9.7 mg/L). No GHB (<3 mg/L) or GBL (<5 mg/L) was detected in beer, distilled alcoholic drinks or fruit juices. The highest GHB concentrations were found to be in red wine, ranging from 4.1 to 21.4 mg/L (mean = 12.6 mg/L). This is compared to white

Table 2
GHB concentrations in red and white wine

Beverage	Country of origin	Alcohol content (%, v/v)	pH	GHB concentration (mg/L)	GBL detected? (>5 mg/L)
Red wine 1	Chile	14	3.70	21.4	Yes
Red wine 2	Australia	14	3.37	10.6	Yes
Red wine 3	Argentina	13	3.43	19.2	Yes
Red wine 4	Australia	14	3.47	15.0	Yes
Red wine 5	Romania	12	3.53	13.5	Yes
Red wine 6	USA	13.5	3.59	15.8	Yes
Red wine 7	USA	13.5	3.24	13.7	Yes
Red wine 8	USA	13.5	3.49	14.9	Yes
Red wine 9	Spain	13.5	3.68	10.2	Yes
Red wine 10	Spain	13	3.58	11.1	Yes
Red wine 11	Spain	12.5	3.46	9.6	Yes
Red wine 12	France	12	3.52	12.1	Yes
Red wine 13	Spain	12.5	3.49	4.1	Yes
Red wine 14	Italy	13	3.25	10.2	Yes
Red wine 15	Argentina	14	3.51	10.8	Yes
Red wine 16	Italy	13	3.47	9.6	Yes
White wine 1	New Zealand	10.5	3.26	8.4	Yes
White wine 2	Australia	12	3.00	5.9	Yes
White wine 3	South Africa	13.5	3.47	8.5	Yes
White wine 4	South Africa		3.51	6.8	Yes
White wine 5	South Africa	12.5	3.51	3.5	Yes
White wine 6	Germany	9.5	3.41	9.6	Yes
White wine 7	Australia	12.5	3.30	5.1	Yes
White wine 8	France	11.5	3.17	5.7	Yes
White wine 9	France	12	3.29	<3	Yes
White wine 10	Italy	12	3.19	3.3	Yes

wine which had an associated GHB range of <3–9.6 mg/L (mean = 5.7 mg/L). For each of the 26 red and white wines analysed, their country of origin, alcohol content and pH was noted (Table 2). There did not appear to be any obvious correlation between these factors and the concentration of GHB found. In theory, a very acidic pH would shift the chemical equilibrium towards GBL, thus lowering the GHB concentration. Although this may be a reason for the reduced GHB concentrations in white wine, the overall similarity of pH values between the red and white wines reduces the likelihood of this explanation. The highest concentration of GHB was detected in a red wine from Chile (21.4 mg/L) and the lowest was from a white wine from France (not detected <3 mg/L). With the additional detection of GHB/GBL in red wine based vermouth and port, it appears red grapes (or the particular production processes associated with them) contain more GHB/GBL or result in the production of more GHB/GBL during the fermentation process than "white" grapes. There was an associated absence of GHB/GBL (<5 mg/L) in both white and red grape juice but this does not necessarily provide any additional evidence for the provenance of the GHB.

With regard the implications of these results, the data indicate there may be interpretative issues when investigating suspected surreptitious GHB administration via the adulteration of wine. Whereby it is likely that naturally occurring GHB will be detected. Due to the observed variation of concentrations even within grape types it is recommended that specific analyses be performed of purchased products of the same type as that implicated in the case. Other beverages do not seem to contain any endogenous GHB and therefore detection (e.g. at concentrations above 10 mg/L) may indicate potential exogenous introduction to the drink. As previously mentioned, in order for an effective dose to be administered it is conceivable that a far higher concentration would be present in the drink and should therefore be easily distinguishable from any naturally occurring GHB. For example, 2 g of GHB in 250 mL of liquid, a large glass of wine, would result in a beverage concentration of 4000 mg/L. Even 0.5 g of GHB in 250 mL would produce a concentration of 1000 mg/L. In the UK, for one pint of beer (~568 mL) 0.5 g of GHB would produce a concentration of ~880 mg/L. However, if attempts have been made by the perpetrator to remove any added GHB (e.g. by rinsing the container), there is still the possibility that low concentrations of residual GHB may be found.

In the case of commercially available drug testing kits for drinks, there are very limited data concerning the limit of detection for any of the drugs purported to be detected, especially GHB. During a study of the capabilities of the enzymic detection of GHB (utilizing GHB dehydrogenase) Bravo et al. indicated a maximal detection limit of 0.037 mg GHB/mL (37 mg/L) [6]. Therefore, under experimental conditions and using purified enzyme, none of the drinks analysed in this study would necessarily provide a "positive" result. However, as the exact chemistry, cross-reactivity and sensitivity of "drink testing kits" is largely unknown and unpublished, the possibility remains that particularly in the case of wines, a "false positive" result may be achieved due to the presence of naturally occurring GHB in the drink itself. Further evaluation of such kits should be performed either by the manufacturers or other researchers in order to better interpret any findings and determine the usefulness of these products.

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